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# Human milk oligosaccharides

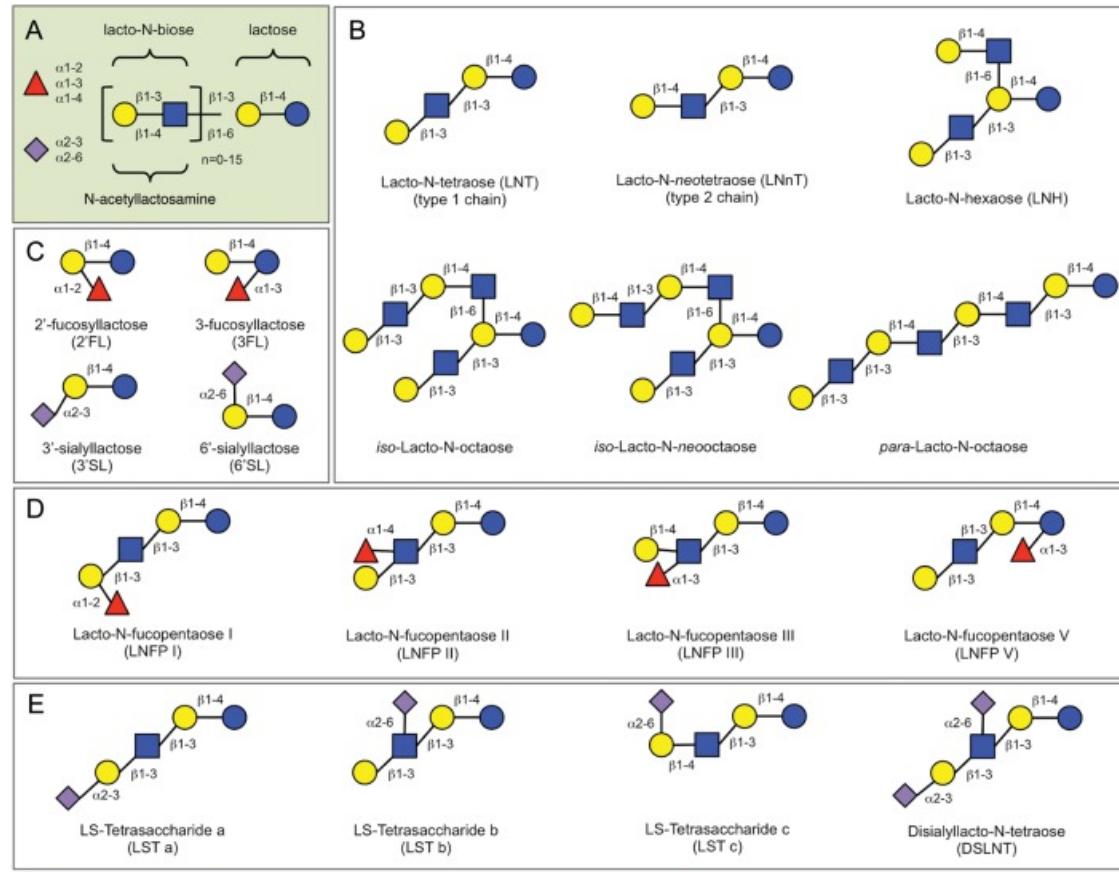
Analysis of mother milk and infant  
faecal samples from the KOALA  
cohort

# Overview

- Introduction to human milk oligosaccharides
- Cohort background
- Experimental setup
- Results

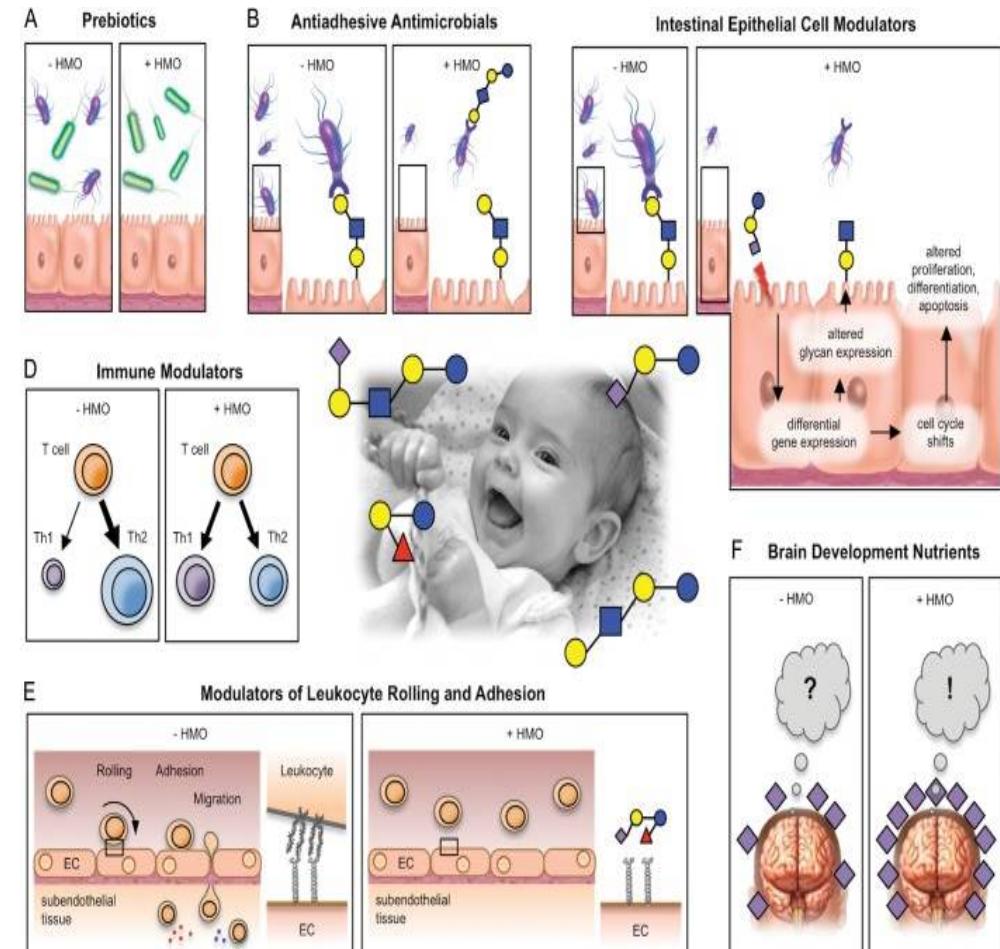


# *hMOS* structures



# Functions of *h*MOS

- Prebiotic
- Anti-adhesive
- Glycomodulating
- Immunomodulating
- Brain development

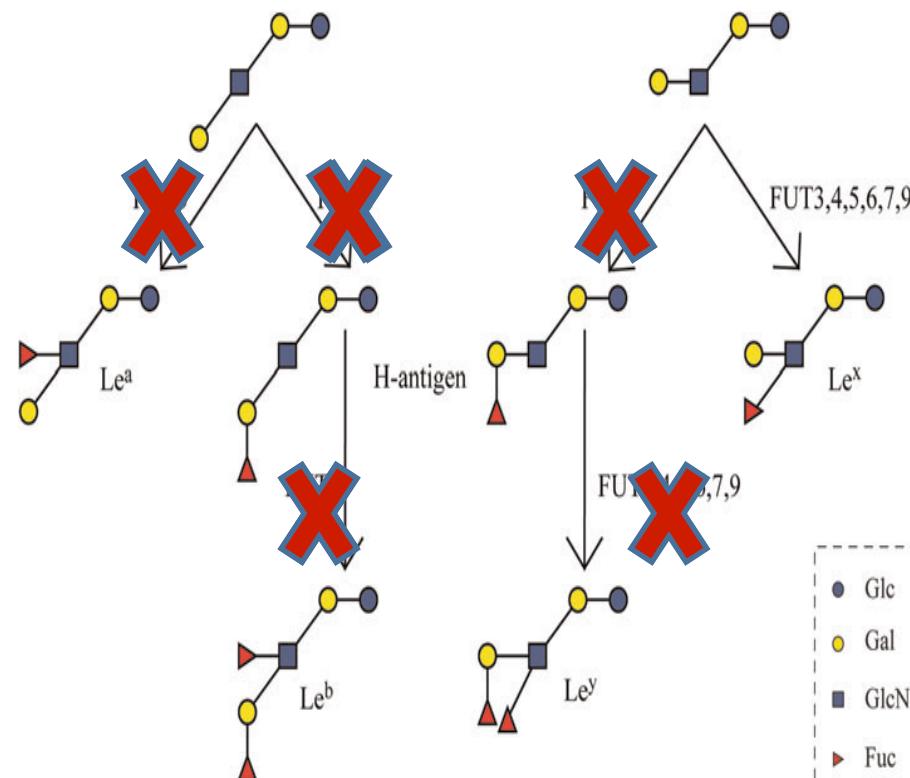


# *hMOS fucosylation*

- Two fucosyltransferases are responsible for secretor and lewis status of the milk
- Fucosyltransferase 2 (FUT2) is responsible for Secretor status
- Fucosyltransferase 3 (FUT3) is responsible for Lewis status
- Milk can be divided in to four groups due to the presence or absence of FUT2 and FUT3

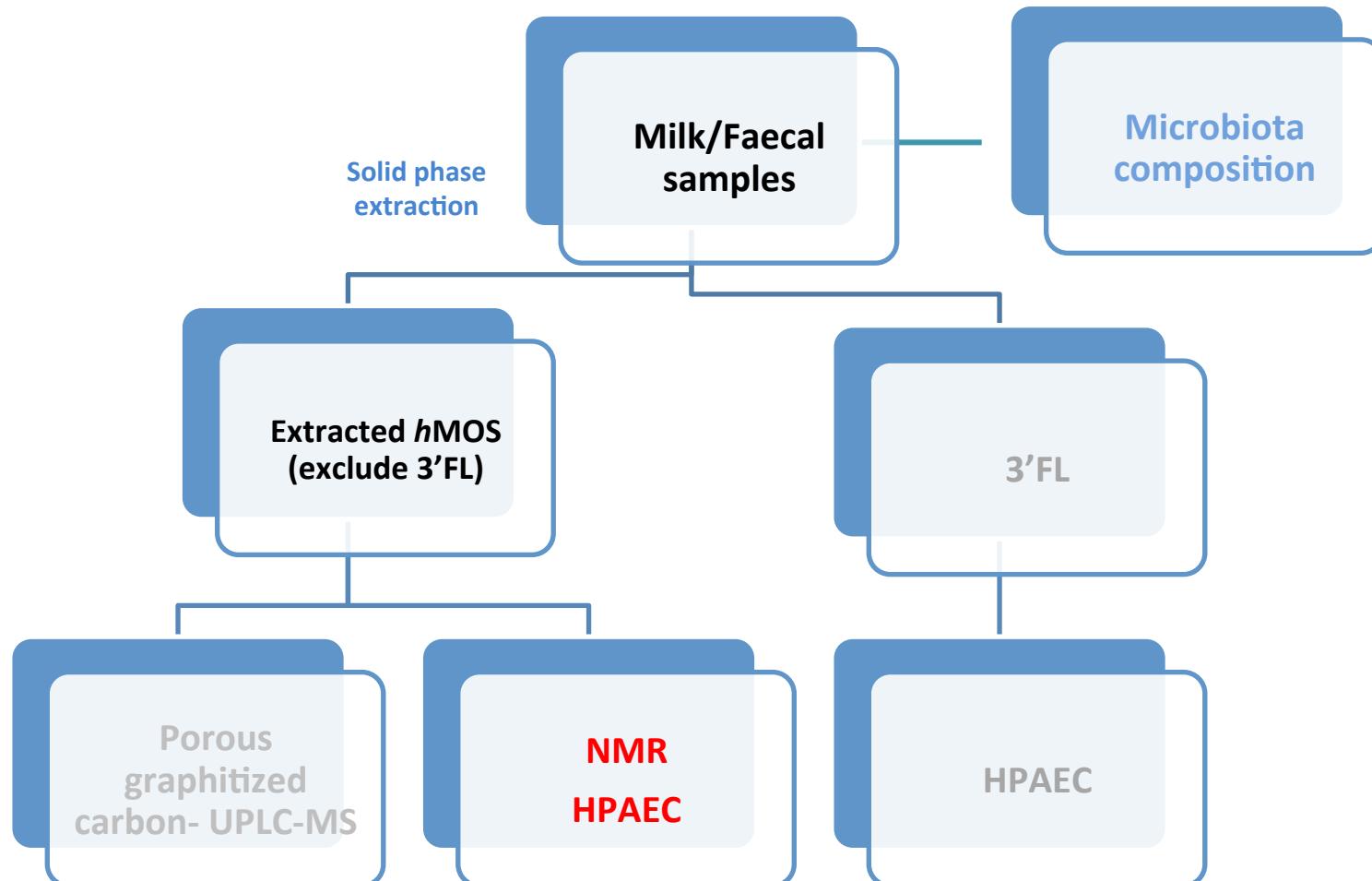
*Group 1 SeLe*      *Group 2 seLe*      *Group 3 Sele*      *Group 4 sele*

Type 1 [ $\beta$ -D-Galp-(1 $\rightarrow$ 3)- $\beta$ -D-GlcNAc-]      Type 2 [ $\beta$ -D-Galp-(1 $\rightarrow$ 4)- $\beta$ -D-GlcNAc-]



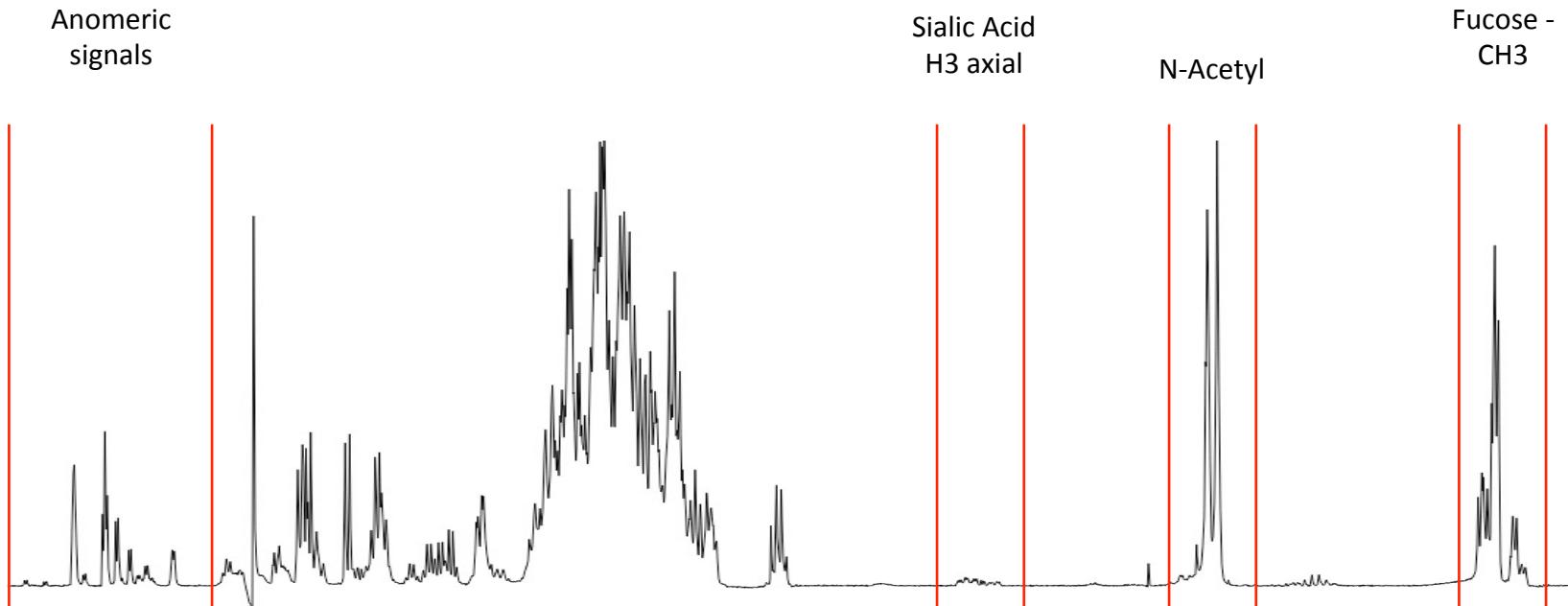
- KOALA birth cohort study in Maastricht collected samples from mothers and babies
- 146 milk samples and 146 corresponding faeces samples
- hMOS isolated by graphitized carbon SPE column
- Isolated hMOS analyses
  - $^1\text{H}$ -NMR spectroscopy (structural reporter groups)
  - HPAEC-PAD profiling
  - PGC-UPLC-MS profiling

# Methods



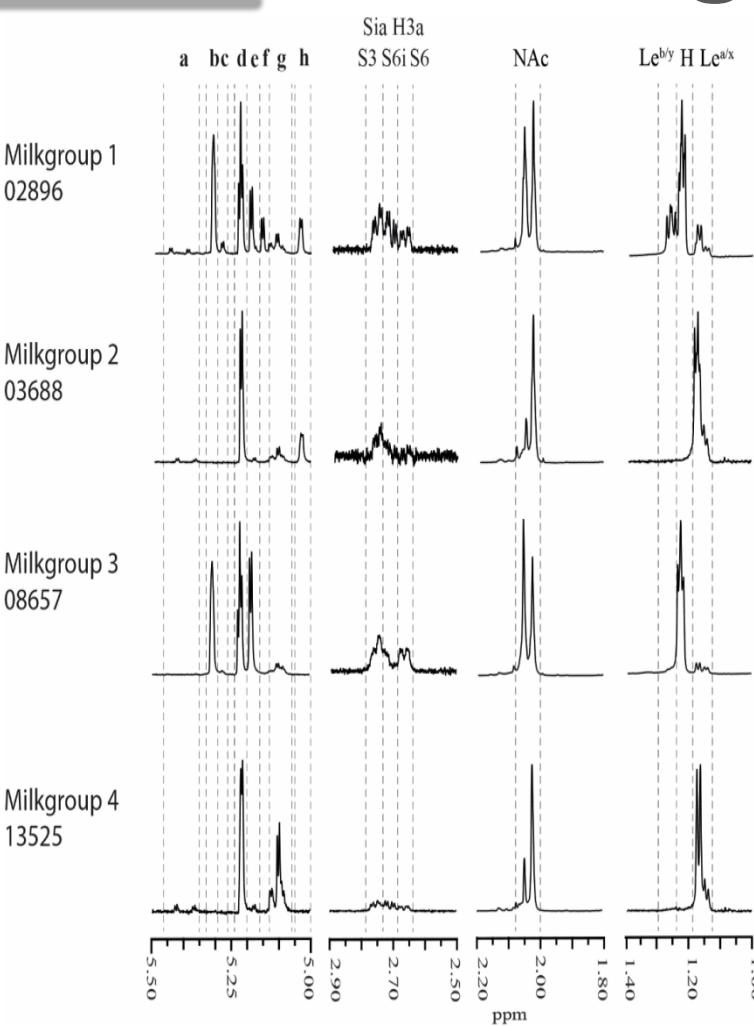
# Structural reporter groups

- Peaks in  $^1\text{H}$ -NMR spectra that are indicative of a specific structural element
- For hMOS we look at four different regions

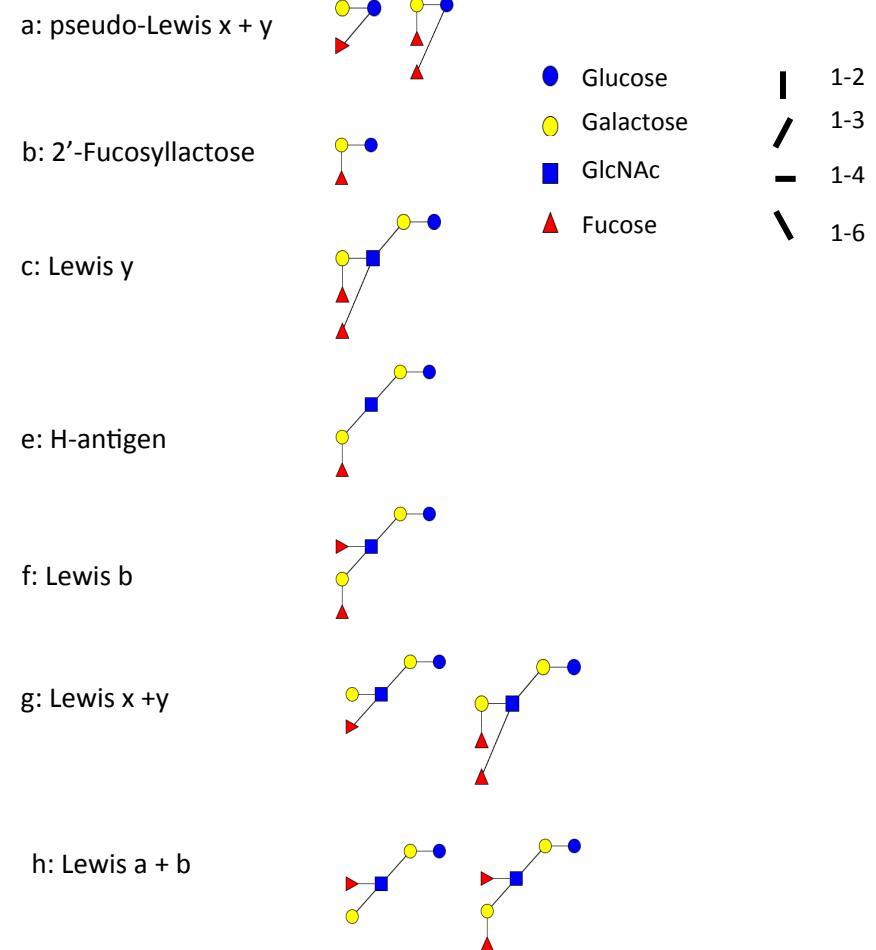




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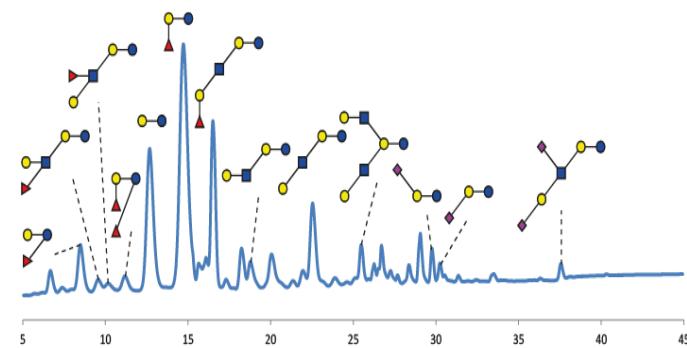
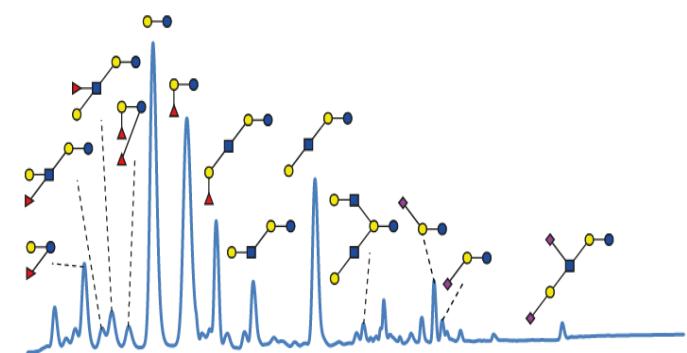
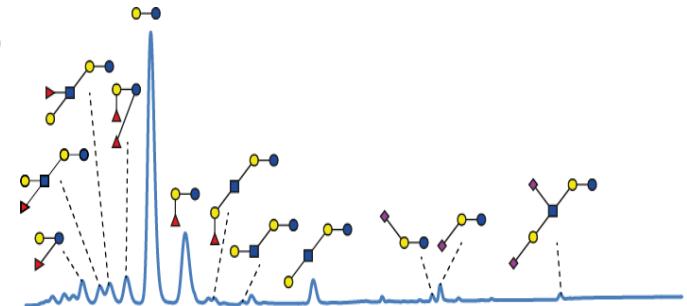


# Milk group classing



# HPAEC

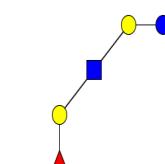
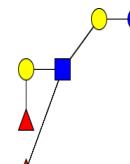
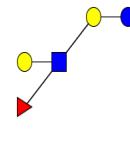
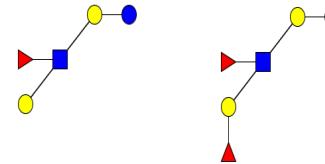
- All milk group 1 samples
- 14 reference structures



# Milk group distribution

Lewis										
Group	a	b	x	y	H	Secret or	Lewis	Numb er	Percent age	Expecte d *
1	✓	✓	✓	✓	✓	+	+	101	69%	69%
2	✓			✓		-	+	32	22%	20%
3				✓	✓	+	-	9	6%	10%
4				✓		-	-	4	3%	1%

Lewis <sup>a</sup>      Lewis <sup>b</sup>      Lewis <sup>x</sup>      Lewis <sup>y</sup>      H-antigen

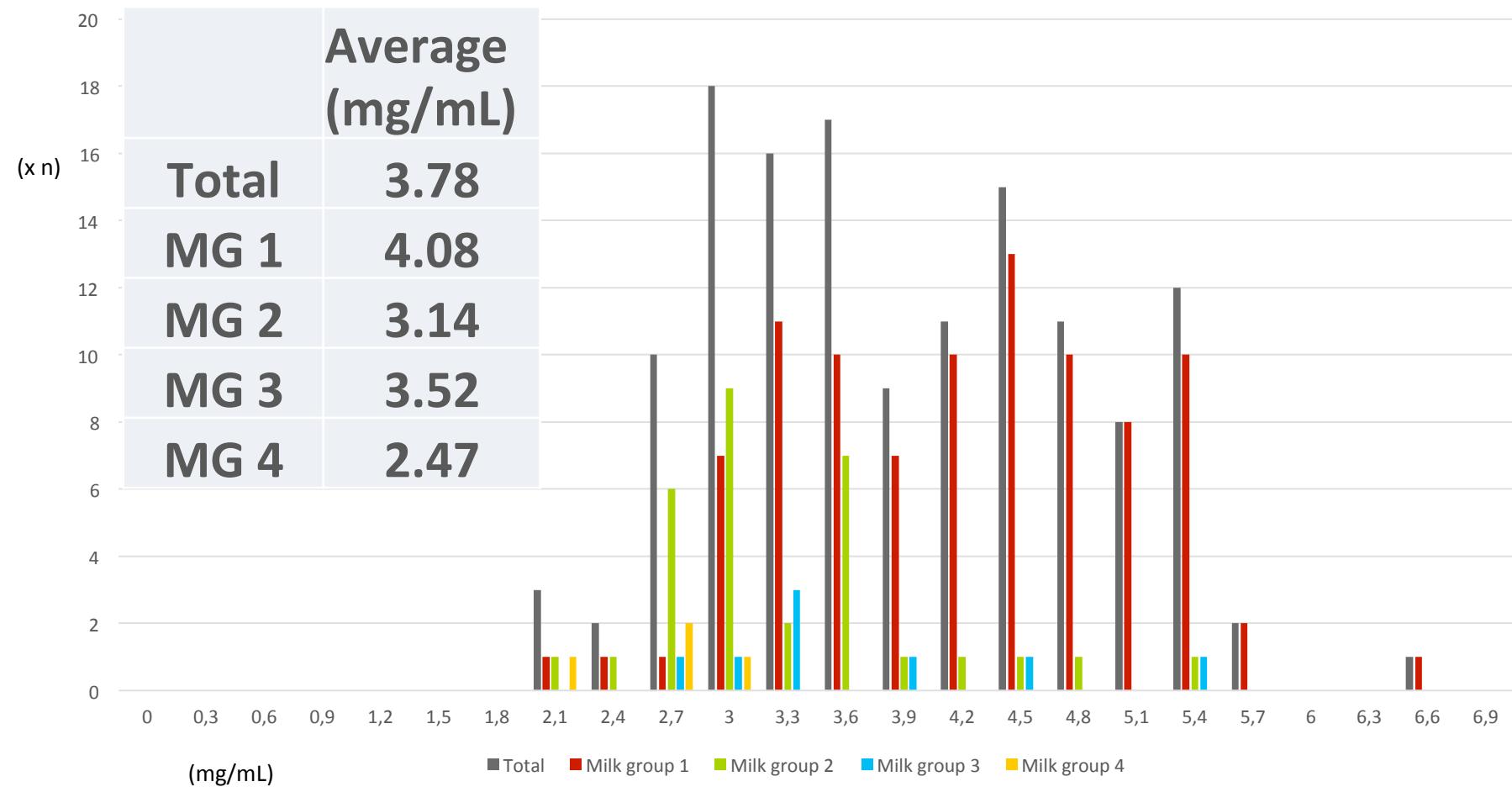


- Glucose
  - Galactose
  - GlcNAc
  - ▲ Fucose
- 1-2
- 1-3
- 1-4
- 1-6

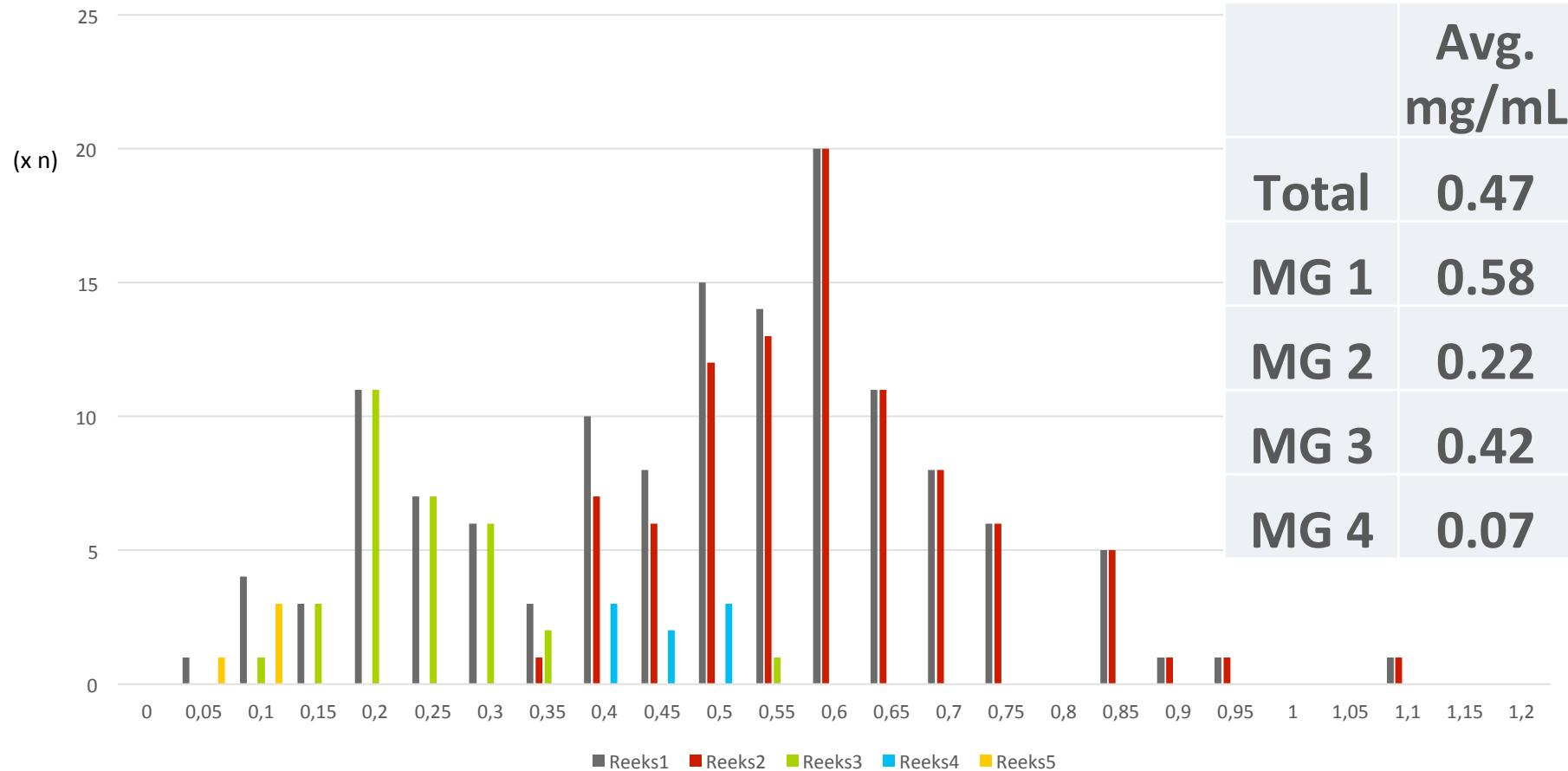


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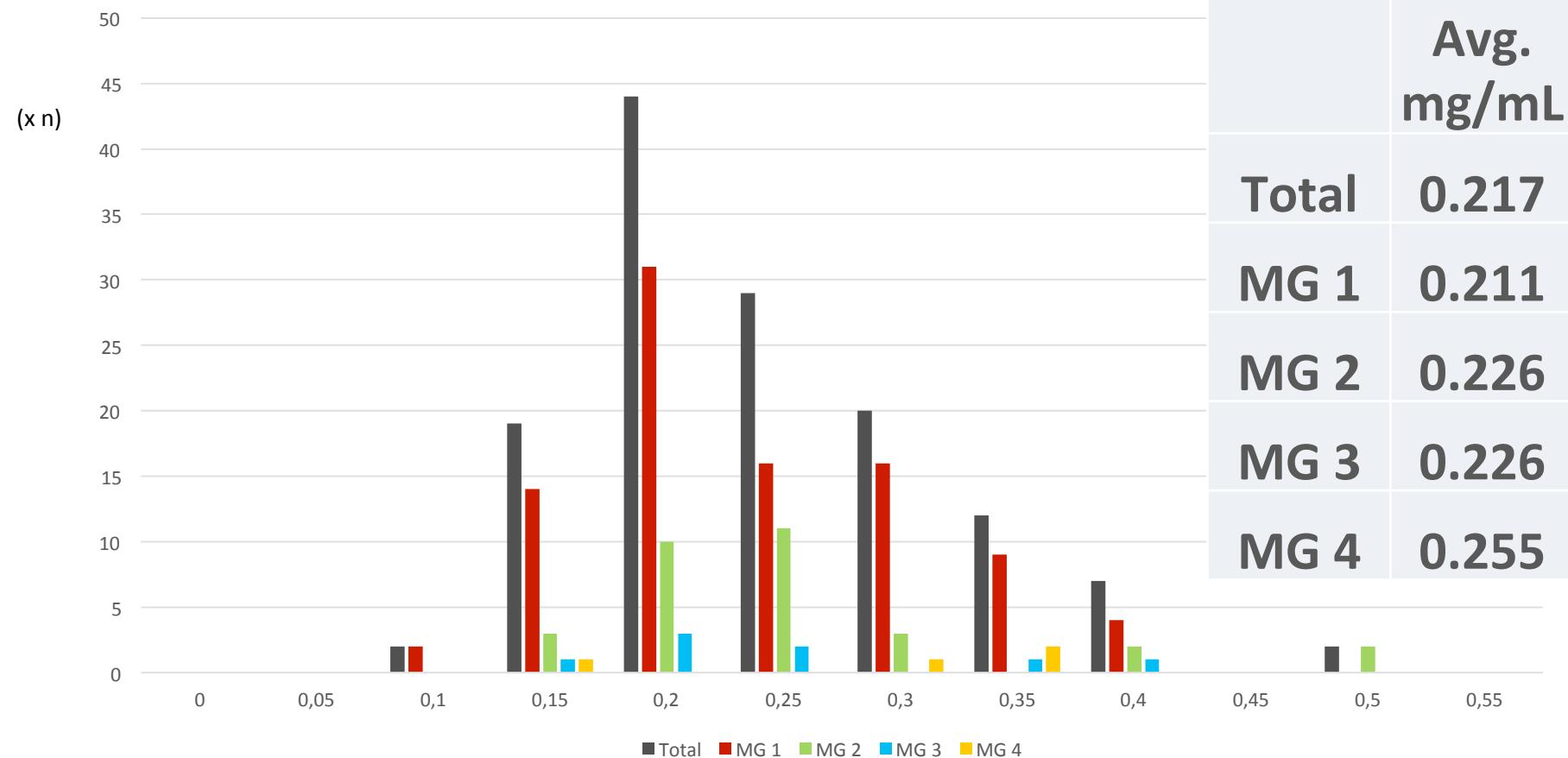
# hMOS levels (mg/mL)



# Fucose distribution

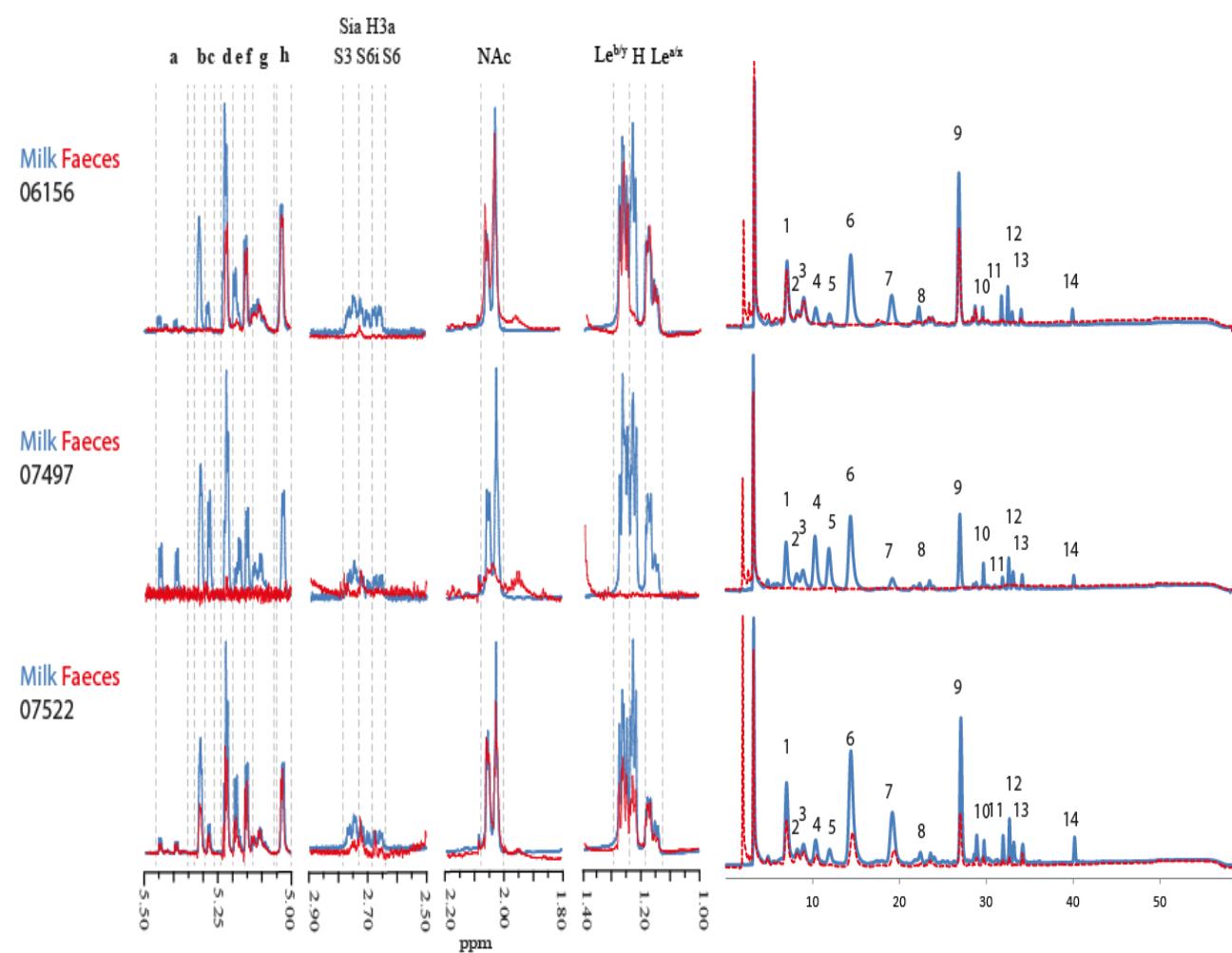


# Neu5Ac distribution

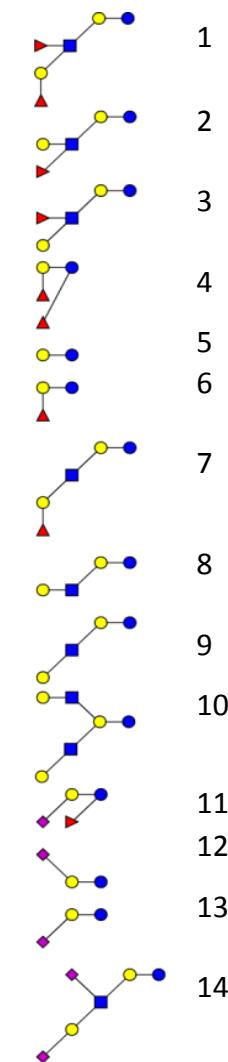




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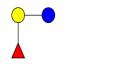
# hMOS consumption



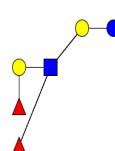


# Consumption patterns

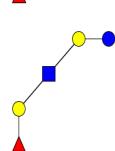
2'-FL



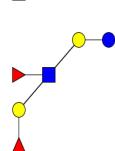
Lewis Y



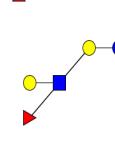
H-antigen



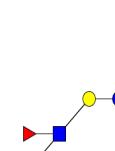
Lewis B



Lewis X



Lewis A



	Milk	Le <sup>a</sup>	Le <sup>b</sup>	Le <sup>x</sup>	Le <sup>y</sup>	2'-FL	H	Faeces	Le <sup>a</sup>	Le <sup>b</sup>	Le <sup>x</sup>	Le <sup>y</sup>	2'-FL	H
2'-FL	<b>10259</b>	0.02	0.06	0.00	0.06	0.67	0.19	<b>10259</b>	0.03	<b>0.62</b>	0.00	<b>0.00</b>	<b>0.00</b>	<b>0.35</b>
Lewis Y	<b>08363</b>	0.09	0.17	0.20	0.07	0.36	0.11	<b>08363</b>	<b>0.12</b>	0.20	0.22	0.05	<b>0.29</b>	0.12
H-antigen	<b>13371</b>	0.25	0.15	0.04	0.06	0.39	0.11	<b>13371</b>	<b>0.11</b>	<b>0.44</b>	<b>0.33</b>	0.05	<b>0.00</b>	0.07
Lewis B	<b>13341</b>	0.10	0.20	0.22	0.04	0.25	0.18	<b>13341</b>	<b>0.00</b>	<b>0.14</b>	<b>0.48</b>	0.00	<b>0.00</b>	<b>0.38</b>
Lewis X	<b>07583</b>	0.08	0.20	0.16	0.07	0.34	0.14	<b>07583</b>	0.10	0.24	0.16	0.08	<b>0.29</b>	0.13
Lewis A	<b>13279</b>	0.05	0.16	0.13	0.07	0.41	0.19	<b>13279</b>	<b>0.11</b>	<b>0.23</b>	<b>0.26</b>	0.04	<b>0.16</b>	0.20
	<b>13336</b>	0.02	0.14	0.18	0.05	0.47	0.14	<b>13336</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
	<b>13261</b>	0.09	0.18	0.17	0.06	0.39	0.11	<b>13261</b>	<b>0.22</b>	<b>0.28</b>	<b>0.49</b>	0.00	<b>0.00</b>	<b>0.01</b>
	<b>09693</b>	0.08	0.22	0.18	0.04	0.26	0.22	<b>09693</b>	<b>0.05</b>	<b>0.50</b>	<b>0.00</b>	0.00	<b>0.00</b>	<b>0.45</b>
	<b>08442</b>	0.08	0.19	0.14	0.04	0.36	0.20	<b>08442</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
	<b>09535</b>	0.00	0.10	0.06	0.08	0.65	0.11	<b>09535</b>	0.00	0.12	<b>0.09</b>	0.05	<b>0.61</b>	0.14
	<b>13501</b>	0.04	0.17	0.12	0.08	0.44	0.15	<b>13501</b>	<b>0.08</b>	0.19	0.14	0.05	<b>0.41</b>	0.13
	<b>13575</b>	0.00	0.00	0.03	0.06	0.60	0.31	<b>13575</b>	0.00	0.00	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>1.00</b>
	<b>08509</b>	0.04	0.16	0.18	0.05	0.36	0.21	<b>08509</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
	<b>13522</b>	0.01	0.17	0.14	0.06	0.35	0.26	<b>13522</b>	0.04	0.17	<b>0.24</b>	0.06	<b>0.31</b>	<b>0.18</b>
	<b>13567</b>	0.04	0.15	0.19	0.03	0.34	0.24	<b>13567</b>	<b>0.07</b>	0.15	0.18	0.02	<b>0.17</b>	<b>0.41</b>
	<b>13416</b>	0.09	0.19	0.18	0.05	0.31	0.19	<b>13416</b>	0.08	<b>0.37</b>	<b>0.00</b>	0.00	<b>0.06</b>	<b>0.49</b>
	<b>13471</b>	0.49	0.00	0.51	0.00	0.00	0.00	<b>13471</b>	<b>0.13</b>	0.00	<b>0.88</b>	0.00	0.00	0.00
	<b>13555</b>	0.01	0.13	0.09	0.06	0.51	0.20	<b>13555</b>	<b>0.10</b>	<b>0.69</b>	0.00	0.00	<b>0.06</b>	<b>0.15</b>
	<b>13504</b>	0.00	0.10	0.07	0.06	0.57	0.20	<b>13504</b>	0.00	0.16	0.09	0.04	<b>0.33</b>	<b>0.38</b>



# Summary consumption

	<b>1</b>		<b>2</b>		<b>3</b>		<b>4</b>	
Total	33	40%	20	64%	1	13%	3	75%
Specific	39	47%	4	13%	5	62%	0	0%
Non specific	11	13%	7	23%	2	25%	1	25%

# Conclusions

- Milk group distribution fits with previous studies
- Fucose level distribution strongly correlated to milk groups
- *h*MOS levels distribution weakly correlated to milk groups
- Neu5Ac levels distribution not correlated to milk groups
- Three profiles of *h*MOS consumption,
- Consumption patterns not correlated to
  - milk groups
  - levels of *h*MOS in milk
- Studies in time could provide better understanding of utilization of *h*MOS



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Geralt ten Kate  
Lubbert Dijkhuizen



Ellen van Leusen  
Cordula Lindner



Maastricht University

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Ilja Arts  
John Penders  
Carel Thijs



KOALA - STUDY



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Gangjie Gu  
Henk Schols



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